

Supporting Information

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SI Text

Protein Preparation. α HL was produced as described in detail ref. 1. In brief, the protein was expressed in the presence of [35 S]methionine in an *Escherichia coli* in vitro transcription and translation (IVTT) system (*E. coli* T7 S30 Extract System for Circular DNA, Catalog no. L1130, Promega). IVTT reactions (100 μ L) containing α HL monomers were incubated with rabbit red blood cell membranes for 1 h at 37 °C to form α HL heptamers. The solution was centrifuged at 25,000 $\times g$ and the pellet containing heptamers was loaded onto a 5% SDS-polyacrylamide gel, which was run for 4 h at 100 V and subsequently vacuum dried for 3–4 h onto Whatman 3M filter paper. The dried gel was exposed to photographic film for 2 h and the developed film was used to locate the position of the heptameric protein in the gel. This region of the gel was excised, rehydrated and crushed in 400 μ L of 10 mM Tris-HCl, pH 8.0, containing 100 μ M EDTA. After 20 min at room temperature, the polyacrylamide was removed by centrifuging the suspension at 25,000 $\times g$ for 7 min at room temperature through a cellulose micro spin column (Microfilteruge tubes, Catalog no. 7016-024, Rainin). Aliquots of the purified protein were stored at –80 °C. The mutant α HL gene was prepared by using a kit for site-directed mutagenesis (QuikChange II XL, Catalog no. 200522-5, Stratagene). The DNA sequence of each gene was verified.

- Cheley S, Braha O, Lu X, Conlan S, Bayley H (1999) A functional protein pore with a "retro" transmembrane domain. *Protein Sci* 8:1257–1267.
- Montal M, Mueller P (1972) Formation of bimolecular membranes from lipid monolayers and study of their electrical properties. *Proc Natl Acad Sci USA* 69:3561–3566.

Planar Bilayer Recordings. Electrical recordings were carried out with a planar lipid bilayer apparatus (2) with a bilayer of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids) formed across an aperture (\approx 100 μ m in diameter) in a 25- μ m-thick polytetrafluoroethylene film (Teflon) (Goodfellow Cambridge, Catalog no. FP301200/10), which separates the apparatus into *cis* and *trans* compartments. Bilayers were formed by first pretreating the aperture with 10 mg mL $^{-1}$ hexadecane in *n*-pentane. Electrolyte solution (0.5 mL: 1 M KCl, 25 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) was added to both compartments. Then, DPhPC in *n*-pentane (10 mg mL $^{-1}$) was added to both compartments. The solvent was allowed to evaporate and the bilayer was formed by lowering and raising the electrolyte level past the aperture.

Under the conditions of the experiments, all of the pores exhibited a stable open-pore current. The current-voltage characteristics of WT pores are weakly rectifying (1, 3). This rectification is lost in E111N/K147N pores (supporting information (SI) Fig. S3). However, this difference is not relevant to the present work, because both pore types have similar open-pore currents at +160 mV, which is the potential at which our experiments were conducted.

- Gu L-Q, et al. (2000) Reversal of charge selectivity in transmembrane protein pores by using non-covalent molecular adapters. *Proc Natl Acad Sci USA* 97:3959–3964.

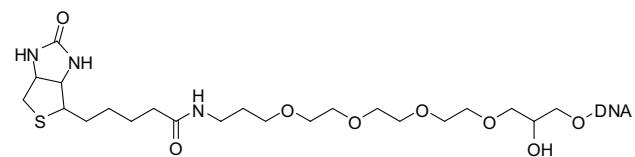


Fig. S1. The chemical structure of the biotin-TEG linker used to biotinylate the 3' terminus of the DNA oligonucleotides. The structure was produced with ChemBioDraw Ultra 11.

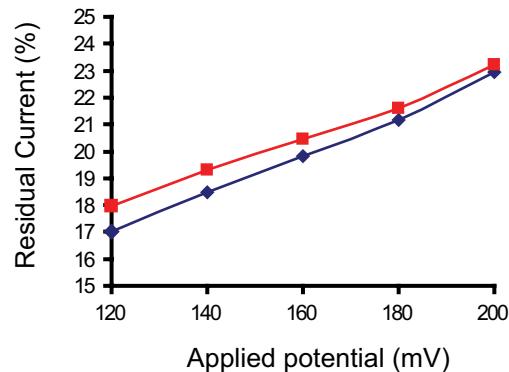


Fig. S2. Voltage dependence of I_{RES} for WT pores threaded with either poly(dA) (red line) or poly(dC) (blue line). The data for the graph were obtained by taking mean values from Gaussian fits to histograms of residual current levels for multiple blockades for each oligonucleotide, at various applied potentials. The standard deviation of the Gaussian fit is shown.

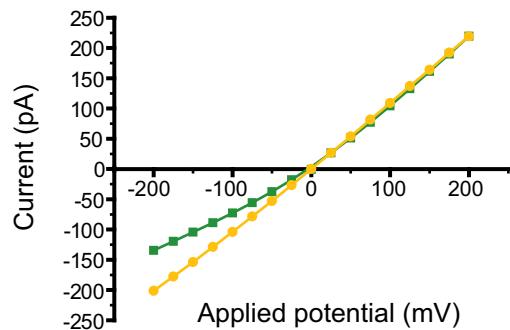


Fig. S3. Typical current-voltage (IV) traces for WT (green) and E111N/K147N (orange) α HL pores, in 1 M KCl, 25 mM Tris-HCl, pH 8.0, containing 0.1 mM EDTA.

Table S1. Residual currents (I_{RES}) for poly(dC) and poly(dA) oligonucleotides immobilized within WT and E111N/K147N pores

Expt.	WT				Expt.	E111N/K147N			
	I_o , pA	$I_{\text{RES}}^{\text{dC}}$, %	$I_{\text{RES}}^{\text{dA}}$, %	ΔI_{RES} , %		I_o , pA	$I_{\text{RES}}^{\text{dC}}$, %	$I_{\text{RES}}^{\text{dA}}$, %	ΔI_{RES} , %
1	163	17.1	17.8	0.7	1	172	37.1	34.1	-3.0
2	172	17.6	18.3	0.7	2	162	36.4	33.9	-2.5
3	187	21.4	21.9	0.5	3	166	35.5	32.6	-2.9
4	170	19.9	20.5	0.7	4	176	37.1	34.1	-3.0
5	169	19.8	20.5	0.6	5	167	36.9	33.8	-3.1
6	163	20.2	20.8	0.6	6	165	36.9	33.9	-3.0
7	172	19.6	20.0	0.4	7	192	37.2	35.3	-1.9
8	173	19.9	20.5	0.6	8	171	35.8	33.5	-2.3
Mean	171	19.4	20.0	0.6	Mean	171	36.6	33.9	-2.7
SD	8	1.4	1.3	0.1	SD	9	0.6	0.7	0.4

The I_o and I_{RES} values given for each oligonucleotide are mean values taken from Gaussian fits to event histograms for individual experiments. ΔI_{RES} is the difference in residual current between the poly(dA) and poly(dC) blockades ($I_{\text{RES}}^{\text{poly(dA)}} - I_{\text{RES}}^{\text{poly(dC)}}$).

Table S2. Summary of residual current levels (I_{RES}) for α HL pores containing poly(dC) and poly(dA) oligonucleotides

DNA immobilization technique	Pore	End of DNA threaded	I_{RES}^{dA} , %	I_{RES}^{dC} , %	ΔI_{RES} , %	Conditions (buffer / applied potential). Experiments conducted between 19 and 25 °C	Reference
Streptavidin–biotin	WT	5'	20.0	19.4	+0.6	1 M KCl, 25 mM Tris-HCl, pH 8.0, with 0.1 mM EDTA / +160 mV	Current work
Streptavidin–biotin	E111N/K147N	5'	33.9	36.6	-2.7	1 M KCl, 25 mM Tris-HCl, pH 8.0, with 0.1 mM EDTA / +160 mV	Current work
Terminal hairpin	WT	5'	21.5	32.0	-10.5	500 mM KCl, 5 mM MOPS, pH 7.5 / +170 mV	1
Terminal hairpin	WT	3'	22.0	31.0	-9.0	500 mM KCl, 5 mM MOPS, pH 7.5 / +170 mV	1
N/A (freely translocating)	WT	Unknown	12.6	13.4	-0.8	1 M KCl, 10 mM Tris-HCl, pH 8.5 / +120 mV	2
Streptavidin–biotin	WT	5'	18.3	17.1	+1.2	1 M KCl, 10 mM Tris-HCl, pH 8, with 1 mM EDTA / +120 mV	3
Streptavidin–biotin	WT	3'	16.0	18.9	-2.9	1 M KCl, 10 mM Tris-HCl, pH 8, with 1 mM EDTA / +120 mV	3

ΔI_{RES} is defined as the difference in residual current between the poly(dA) and poly(dC) blockades ($I_{RES}^{poly(dA)} - I_{RES}^{poly(dC)}$).

1. Ashkenasy N, Sánchez-Quesada J, Bayley H, Ghadiri MR (2005) Recognizing a single base in an individual DNA strand: A step toward nanopore DNA sequencing. *Angew Chem Int Ed Engl* 44:1401–1404.
2. Meller A, Nivon L, Brandin E, Golovchenko J, Branton D (2000) Rapid nanopore discrimination between single polynucleotide molecules. *Proc Natl Acad Sci USA* 97:1079–1084.
3. Purnell RF, Mehta KK, Schmidt JJ (2008) Nucleotide identification and orientation discrimination of DNA homopolymers immobilized in a protein nanopore. *Nano Lett* 8:3029–3034.

Table S3. Residual currents (I_{RES}) for poly(dC) oligonucleotides containing a stretch of 5 consecutive adenine nucleotides immobilized within WT and E111N/K147N pores

Oligo i–v	WT					Oligo i–v	E111N/K147N				
	I_O , pA	I_{RES}^{i-v} , %	$I_{RES}^{poly(dC)}$, %	n	ΔI_{RES} , %		I_O , pA	I_{RES}^{i-v} , %	$I_{RES}^{poly(dC)}$, %	n	ΔI_{RES} , %
i	168 ± 2	19.3 ± 0.7	19.3 ± 0.7	3	0.0 ± 0.0	i	158 ± 1	35.8 ± 1.8	35.8 ± 1.8	3	0.0 ± 0.0
ii	171 ± 4	19.7 ± 0.8	19.3 ± 0.7	3	0.4 ± 0.2	ii	162 ± 7	35.2 ± 0.1	36.8 ± 0.1	3	-1.6 ± 0.1
iii	178 ± 13	22.3 ± 1.3	21.1 ± 1.1	5	1.2 ± 0.3	iii	169 ± 8	39.0 ± 1.2	37.5 ± 0.9	4	1.5 ± 0.4
iv	175 ± 11	20.1 ± 1.3	21.1 ± 1.5	3	-1.0 ± 0.2	iv	171 ± 7	35.3 ± 0.5	37.5 ± 0.9	3	-2.2 ± 0.5
v	166 ± 14	21.3 ± 1.3	21.3 ± 1.3	3	0.0 ± 0.0	v	168 ± 8	37.8 ± 1.4	37.8 ± 1.4	3	0.0 ± 0.0

The I_O and I_{RES} values shown are the mean values from n experiments. ΔI_{RES} is the difference in residual current between each A₅ oligonucleotide (i–v) (Fig. 2A) and poly(dC) ($I_{RES}^{A_5\text{oligo}} - I_{RES}^{\text{poly(dC)}}$). The errors given are standard deviations.

Table S4. Residual currents (I_{RES}) for poly(dC) and oligonucleotides that contain a single adenine nucleotide

Position of adenine	WT					Position of adenine	E111N/K147N				
	I_o , pA	$I_{\text{RES}}^{\text{A}1}$, %	$I_{\text{RES}}^{\text{dC}}$, %	n	ΔI_{RES} , %		I_o , pA	$I_{\text{RES}}^{\text{A}1}$, %	$I_{\text{RES}}^{\text{dC}}$, %	n	ΔI_{RES} , %
7	167 ± 1	20.4 ± 0.4	20.2 ± 0.6	3	0.3 ± 0.2	7	169 ± 10	37.0 ± 0.4	37.0 ± 0.4	3	0.0 ± 0.0
8	170 ± 4	20.1 ± 0.4	19.6 ± 0.4	3	0.6 ± 0.1	8	163 ± 2	34.9 ± 3.9	34.9 ± 3.9	3	0.0 ± 0.0
9	169 ± 3	20.5 ± 0.5	19.9 ± 0.4	3	0.6 ± 0.1	9	163 ± 1	34.3 ± 3.8	34.9 ± 3.7	3	-0.6 ± 0.1
10	173 ± 2	20.3 ± 0.2	20.0 ± 0.2	4	0.3 ± 0.0	10	175 ± 4	36.1 ± 0.4	37.0 ± 0.4	3	-0.9 ± 0.1
11	168 ± 8	20.0 ± 0.2	20.0 ± 0.2	3	0.0 ± 0.0	11	165 ± 5	36.6 ± 0.1	37.2 ± 0.1	3	-0.6 ± 0.1
12	173 ± 12	20.1 ± 0.1	20.0 ± 0.2	3	0.1 ± 0.1	12	164 ± 6	35.0 ± 2.2	35.0 ± 2.2	3	0.0 ± 0.0
13	168 ± 6	20.4 ± 0.3	19.8 ± 0.3	3	0.5 ± 0.1	13	164 ± 6	37.0 ± 1.8	36.3 ± 1.7	3	0.7 ± 0.1
14	170 ± 8	20.5 ± 0.7	19.7 ± 0.6	3	0.8 ± 0.1	14	167 ± 9	37.1 ± 1.5	35.5 ± 1.4	3	1.6 ± 0.1
15	172 ± 5	20.7 ± 0.3	20.0 ± 0.3	3	0.6 ± 0.1	15	164 ± 3	38.4 ± 2.2	36.5 ± 2.2	3	1.9 ± 0.1
16	170 ± 5	19.9 ± 0.2	20.0 ± 0.1	3	-0.1 ± 0.1	16	161 ± 5	37.4 ± 1.3	36.4 ± 1.2	3	1.0 ± 0.2
17	172 ± 5	19.3 ± 0.4	19.8 ± 0.4	3	-0.4 ± 0.0	17	165 ± 3	37.0 ± 0.3	36.9 ± 0.1	3	0.2 ± 0.3
18	171 ± 6	20.2 ± 0.9	20.5 ± 0.9	3	-0.3 ± 0.1	18	165 ± 4	36.2 ± 0.3	37.0 ± 0.4	3	-0.9 ± 0.0
19	172 ± 5	20.0 ± 0.3	20.0 ± 0.3	3	0.0 ± 0.0	19	170 ± 17	36.1 ± 1.6	36.6 ± 1.5	3	-0.5 ± 0.1
20	173 ± 7	20.5 ± 0.9	20.5 ± 0.9	3	0.0 ± 0.0	20	166 ± 5	35.9 ± 1.3	35.9 ± 1.3	3	0.0 ± 0.0

The position of the adenine in the A₁ nucleotide (nucleotides 7–20) is numbered relative to the 3'-biotin tag. The I_o and I_{RES} values are the mean values from n experiments. ΔI_{RES} is defined as the difference in residual current between an A₁ oligonucleotides and poly(dC) ($I_{\text{RES}}^{\text{A}1\text{oligo}} - I_{\text{RES}}^{\text{dC}}$). The errors given are standard deviations.

Table S5. Sequences of the oligonucleotides used in this article

5'-CCBtn-3'
5'-AAAAAAAAAAAAAAAABtn-3'
5'-CCBtn-3'
5'-CCAAABtn-3'
5'-CCAAACCCCBtn-3'
5'-CCBtn-3'
5'-ACTACCTAGTTACGTAATCCATCTGAACAATGCAGCATTBtn-3'
5'-ACTACCTAGTTACGTAATCCATCTGTACAATGCAGCATTBtn-3'
5'-ACTACCTAGTTACGTAATCCATCTGGACAATGCAGCATTBtn-3'
5'-ACTACCTAGTTACGTAATCCATCTGCACAATGCAGCATTBtn-3'

Btn represents the 3'-biotin-TEG tag and linker (Fig. S1).